

# UNCLASSIFIED

AD NUMBER
AD860408
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; SEP 1969. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Releases Branch/TID, Frederick, MD. 21701.
AUTHORITY
Biological Defense Research Lab ltr dtd 29 Sep 1971

THIS PAGE IS UNCLASSIFIED

AD

TECHNICAL MANUSCRIPT 556

A PLAQUE ASSAY  
FOR RICKETTSIA TSUTSUGAMUSHI

Joseph E. McDade

Peter J. Gerone

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Dept. of Army, Fort Detrick, ATTN: Technical Release Branch/TIO. Frederick, Maryland 21701

SEPTEMBER 1969

DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

1

Reproduction of this publication in whole or in part is prohibited except with permission of the Commanding Officer, Fort Detrick, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for United States Government purposes.

#### DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

#### DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

EXEMPTION NO.	
DDC	WHITE SECTION <input type="checkbox"/>
UNANNOUNCED	DIFF. SECTION <input checked="" type="checkbox"/>
JUSTIFICATION	<input type="checkbox"/>
BY	
DISTRIBUTION/AVAILABILITY CODES	
DIST.	AVAIL. AND/OR SPECIES

21

DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 556

A PLAQUE ASSAY FOR RICKETTSIA TSUTSUGAMUSHI

Joseph E. McDade

Peter J. Gerone

Virus & Rickettsia Division  
BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602A059

September 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

#### ABSTRACT

The plaque assay procedure recently developed for the typhus and spotted fever groups of rickettsiae is also successful with scrub typhus rickettsiae.

### A PLAQUE ASSAY FOR RICKETTSIA TSUTSUGAMUSHI\*

Recent reports<sup>1,2</sup> have demonstrated the success of a plaque technique using several rickettsiae from the spotted fever and typhus groups. The experiments reported in this paper demonstrate the applicability of the technique to strains of scrub typhus, Rickettsia tsutsugamushi.

Several vials each of the Karp, Gilliam, and Kato strains of Rickettsia tsutsugamushi (20% yolk sac suspensions in SP-G buffer<sup>3</sup>) were generously supplied by Dr. Bennett L. Elisberg, Walter Reed Army Institute of Research, Washington, D.C. These strains were in their 47th, 136th, and 89th yolk sac passages, respectively. To expand the volume of working seed material, one vial of each strain was thawed, diluted tenfold in sucrose phosphate (SP 25) buffer,<sup>4</sup> dispensed in ampoules, and stored at -65 C. This diluted and refrozen material was used exclusively for plaquing experiments and was not used in any animal tests.

For the plaque assay, 24-hour chick embryo primary monolayers were infected with serial tenfold dilutions, prepared in brain heart infusion broth, of the three strains of R. tsutsugamushi. The infected monolayers were overlaid with 5 ml of medium 199 (5% calf serum) containing 0.5% agarose and incubated at 32 C for 10 days. Three milliliters of a second overlay were placed over the initial overlay after 10 days, and incubation at 32 C was continued for an additional 7 days. Plaques were stained with an overlay containing neutral red as described previously.<sup>1</sup>

The plaque morphology of the three strains of R. tsutsugamushi is shown in Figure 1. The plaques formed by the scrub typhus rickettsiae (1 to 2 mm) are quite similar morphologically to the typhus group plaques shown earlier.<sup>1</sup> However, the scrub typhus organism requires a far longer incubation period (17 versus 10 days) than other typhus-group organisms before plaques appear.

The plaque titer was compared with the animal 50% lethal dose (LD<sub>50</sub>) and 50% infectious dose (ID<sub>50</sub>) values. LD<sub>50</sub> were determined by injecting groups of ten 16- to 18-g male Swiss mice (Fort Detrick strain) with 0.2 ml of serial tenfold dilutions, prepared in SP 25 buffer, of the original seed materials. Deaths were recorded daily for 25 days. ID<sub>50</sub> determinations were made by challenging the survivors on the 26th day with a 1,000 LD<sub>50</sub> dose (0.2 ml) of the Karp strain; survivors were presumed to have been previously infected by the initial dose of inoculum. The LD<sub>50</sub> and ID<sub>50</sub> values were calculated by the Reed-Muench method.

---

\* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.



FIGURE 1. Plaques Formed by Various Strains of *Rickettsia tsutsugamushi*.  
A. Left to right,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilutions of the Karp strain.  
B. Left to right,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilutions of the Gilliam strain.  
C. Left to right,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  dilutions of the Karo strain.  
At far right in each photo is an uninfected control.

A comparison of the plaque titers and the mouse LD<sub>50</sub> and ID<sub>50</sub> titers is shown in Table 1. These data show that the plaque titration method is more sensitive than the mouse titration performed in our laboratory. Elisberg\* previously obtained ID<sub>50</sub> values greater than 8.0 log<sub>10</sub>/ml with the same seed pools of these three strains. However, he employed a different strain of mice in his tests (Charles River ICR certified pathogen-free Swiss mice) that may be more sensitive to these organisms than the strain we used.

TABLE 1. COMPARISON OF PLAQUE TITERS OF SEVERAL STRAINS OF RICKETTSIA TSUTSUGAMUSHI WITH LD<sub>50</sub> AND ID<sub>50</sub> TITERS IN SWISS MICE

Strain	LD <sub>50</sub> log <sub>10</sub> /ml	ID <sub>50</sub> log <sub>10</sub> /ml	Plaque Titer, log <sub>10</sub> /ml
Karp	5.98	6.03	7.54
Gilliam	≤5.2	5.7	7.39
Kato	≤5.2	≤5.26	5.97

Using this procedure we have thus far<sup>1,2</sup> been successful in plaquing every species of Rickettsia that we have tested. The results of this study and of previous studies suggest that the plaque assay procedure may be appropriate for plaquing all rickettsiae and should greatly facilitate their study.

\* Personal communication.

LITERATURE CITED

1. McDade, J.E.; Stakebake, J.R.; Gerone, P.J. 1969. A plaque assay for several species of Rickettsia. J. Bacteriol. (In press).
2. Weinberg, E.H.; Stakebake, J.R.; Gerone, P.J. 1969. Plaque assay for Rickettsia rickettsi. J. Bacteriol. 98:398-402.
3. Bovarnick, Marianna R.; Miller, Judith C.; Snyder, John C. 1950. The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae. J. Bacteriol. 59:509-522.
4. Weiss, E.; Rees, H.B., Jr.; Hayes, J.R. 1967. Metabolic activity of purified suspensions of Rickettsia rickettsi. Nature 213:1020-1022.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified
3. REPORT TITLE		2b. GROUP
A PLAQUE ASSAY FOR <u>RICKETTSIA TSUTSUGAMUSHI</u>		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (First name, middle initial, last name)		
Joseph E. McDade Peter J. Gerone		
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
September 1969	9	4
8a. CONTRACT OR GRANT NO.	8b. ORIGINATOR'S REPORT NUMBER(S)	
B. PROJECT NO. 1B562602A059	Technical Manuscript 556	
c.	8d. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
d.	CMs 6587	
10. DISTRIBUTION STATEMENT		
Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.		
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY
		Department of the Army Fort Detrick, Frederick, Maryland, 21701
13. ABSTRACT		
<p>✓ The plaque assay procedure recently developed for the typhus and spotted fever groups of rickettsiae is also successful with scrub typhus rickettsiae.</p>		
14. Key Words		
<u>Rickettsia tsutsugamushi</u> Plaque Assay Scrub typhus Rickettsiae		

DD FORM 1473

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified

Security Classification